Deposition Process of Polysaccharides with the Development of Japanese Walnut Xylem

AUTHOR(S):

Takabe, Keiji; Fujita, Minoru; Tanaka, Yoshiaki; Harada, Hiroshi

CITATION:

Takabe, Keiji ...[et al]. Deposition Process of Polysaccharides with the Development of Japanese Walnut Xylem. 京都大学農学部演習林報告 1984, 56: 234-240

ISSUE DATE:

1984-11-30

URL:

http://hdl.handle.net/2433/191792

Deposition Process of Polysaccharides with the Development of Japanese Walnut Xylem

Keiji TAKABE, Minoru FUJITA, Yoshiaki TANAKA and Hiroshi HARADA

オニグルミの木部形成にともなう木材多糖の堆積過程

高部　圭司・藤田　稔・田中　克明・原田　浩

Résumé

Thin-layer-chromatography densitometry was applied to investigate the changes of the absolute amount of sugar with the xylem development of Japanese walnut (*Juglans Sieboldiana* Maxim.). The differentiating xylem was successively fractionated from the cambium side to the pith side. Each fraction was extracted with reagents to remove lipids and starch. After hydrolyzing each fraction, the hydrolysates were separated by thin-layer-chromatography. Aqueous solution of ethylenediamine sulfate was sprayed on the TLC-plate, and the fluorescent light of each spot in each fraction was measured by TLC-scanner.

Cellulose deposits continuously from the S_1 stage to the S_3 stage. Glucuronoxylan deposits in the same manner as cellulose, although the amount of the former is less than that of the latter. On the other hand, the deposition of glucomannan occurs behind the cellulose and glucuronoxylan depositions, that is, it occurs from the later part of S_2 stage to the S_3 stage. Arabinose and galactose do not deposit during the secondary wall formation stage, although they deposit during the primary wall formation stage.

要　旨

オニグルミの木部形成にともなう木材多糖の絶対量の変化を薄層クロマトグラフィー・デンシトメトリーで調べた。すなわち分化中木部を形成層側から髄側へ順次分け取り，種々の抽出処理をし酸加水分解した後，中和・濃縮し，薄層クロマトグラフィーで糖を分離した。そしてエチレンジアミン硫酸塩を噴霧し各糖のスポットの蛍光の強さを測定して定量した。

セルロースは S_1 形成期から S_3 形成期まで継続的に堆積する。グルクロノキシランもセルロースとほぼ同様な堆積の仕方を示す。グルコマンナンはセルロースやグルクロノキシランの堆積よりもやや遅れ，S_2 形成後期から S_3 形成期にかけて堆積する。アラビノースやガラクトースは一次壁形成期に堆積するが二次壁形成期には壁に供給されないものと思われる。

1. INTRODUCTION

The investigation of the process of wood cell wall formation by means of chemical analysis provides some information about the interrelations among cellulose, hemicelluloses and lignin in the cell wall. It also provides an important information about the chemical composition of each cell wall layer.

Meier and co-worker[1)2)] investigated the distribution of polysaccharides in the cell wall of Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*) and Silver birch (*Betula verrucosa*). They made radial sections, which contained cambium and differentiating xylem, observed the sections between crossed nicols in order to check the differentiating stages, and excised each of sections into several fractions by a micromanupulator. After hydrolyzing each fraction, they examined the sugar composition by paper chromatography. Furthermore, they measured the area of cell wall layers on the electron microscopic photograph of transverse sections of tracheids or fibers. On the basis of the proportion of cell wall layer and the sugar composition of each fraction, they roughly showed the sugar composition of each cell wall layer after the complicate calculations. Although these studies were excellent in those days, the distribution of polysaccharides in the cell wall should be reexamined because of the poor reliability of paper chromatography to determine the neutral sugars. In addition, the authors believe that the calculation of the sugar composition in each cell wall layer from the increment of the absolute amount of sugars with the cell wall thickening is the better method than the one carried out by Meier and co-worker[1)2)].

In recent years, the authors fractionated successively the differentiating xylem of cryptomeria (*Cryptomeria japonica* D. Don), which was embedded in methacrylate resin, and examined the changes of the absolute amount of neutral sugars with the development of cell wall by means of gas-liquid-chromatography. They indicated that the depositing stages are different between cellulose and hemicelluloses. These results will be the fundamental data to express the distribution of polysaccharides in the cell wall, when it will become apparent whether hemicellulose deposits appositionally or internally.

These studies were carried out by paper-chromatography or gas-liquid-chromatography. The former is less reliable to determine the sugar composition than any other analytical methods, and needs much more time to separate the sugars. On the other hand, the latter has the greatest reliability among the analytical methods of sugar, and the time of analysis is relatively short. Therefore, numerous investigators employ the gas-liquid-chromatography in determining neutral sugars. The gas-liquid-chromatography, however, needs the conversion from the sugars to their corresponding alditol acetates. As the conversion is tedious, it is desired to develop a simpler method.

In recent years, Iwakawa and co-workers[4)5)] developed a thin-layer-chromatography-densitometry for determining various sugars. This method has a relatively great reliability in determination of sugar, and the time of analysis is extremely short. In addition, the lower limit of detection is 40 ng of sugar.

In this study, the authors examined the possibility of determining the neutral sugars

contained in wood by the thin-layer-chromatography-densitometry. Furthemore, the authors investigated the deposition process of woody polysaccharides with the development of Japanese walnut xylem.

2. MATERIALS AND METHODS

2.1 Fractionation of differentiating xylem of Japanese walnut

Japanese walnut (*Juglans Sieboldiana* Maxim.) (D.B.H.=ca. 50 cm), which grew at Ashu (Kyoto Prefecture) Experimental Forest of Kyoto University, was used. A large block, which contained cambium and differentiating xylem, was obtained at the height of breast, and fixed in 3% glutaraldehyde over night. It was excised into small blocks (12×8×10 mm; longitudinal, tangential, and radial directions) and fixed again in 3% glutaraldehyde over night. The specimens were washed with M/15 phosphate buffer, dehydrated through a graded ethanol series, and embedded in methacrylate resin (methyl methacrylate: butyl methacrylate, 1/1).

Differentiating xylem in the embedded speciman was fractionated according to the method of Takabe and co-workers[3]. In this study, serial 5 μm thick tangential sections were cut, and thirty sections were collected in a vial. Ten series of fractionated sections were prepared.

2.2 Sugar analysis

Each fraction was extracted with acetone (room temperature, 1 hour, 3 times), chloroform-methanol (1:1) (room temperature, 10 hour, 1 time), and hot water (3 hour, 1 time), followed by the treatment with 0.05% 4× crystalline *Bacillus subtillis* α-amylase in M/20 phosphate buffer (pH. 6.9) for 24 hour at room temperature. After washing, each fraction was added 100 μl of 72% sulfuric acid and gently shaken for 2 hour at room temperature. Then, the solution was diluted to 4% sulfuric acid by adding distilled water, and heated at 120 °C for 2 hour. After hydrolysis, 150 μg of 2-deoxy-D-glucose was added as an internal standard to each solution, and the solution was filtered through Whatman filter paper No. 3 to remove the insoluble materials. The filtrate was passed through Dowex 1×8 (carbonate from), and dried. The hydrolysate was redissolved by adding 100 μl of distilled water, and 1 μl of the solution was spotted on TLC-plate. Thin-layer-chromatography-densitometory was carried out according to the method of Iwakawa and co-workers[4][5], and determinations of the amount of sugar are described in 3.1.

2.3 Correction for the lumen space of vessel.

The area of vessel elements and fibers in each fraction was measured on the photograph of transverse section (Figure 3), which was cut before fractionation. The amount of sugars in each fraction were corrected with the following equation.

The amount of sugar=value by densitometory×(Af+Av)/Af

Af, area of fibers

Av, area of vessel elements

3. RESULTS

3.1 Preparation of calibration curve

Figure 1 shows the calibration curve of glucose when 2-deoxy-D-glucose is used as the internal standard. When ratio of the weight of glucose to that of 2-deoxy-D-glucose is low, an approximately linear relationship exsists between the ratio of the weight and that of the integrated value in densitometory. Whereas, the plot of the ratio of the weight against that of the integrated value becomes nonlinear with the increment of the ratio of the weight. Figure 2 shows the relationship between log [ratio of the weight] and log [ratio of the integrated value]. The data presented in Figure 2 form a straight line in the wide range of sugar concentration. The same results were obtained for other sugars: galactose, mannose, xylose, and arabinose. Consequently, the amount of sugar contained in the fraction were calculated with the equations (Table 1), which were obtained from the calibration curves.

Table 1. Equations for the determination of the sugars

Glc.	$\log y = 0.798 \log x + 0.104$
Gal.	$\log y = 0.880 \log x + 0.121$
Man.	$\log y = 0.884 \log x + 0.220$
Ara.	$\log y = 0.914 \log x + 0.130$
Xyl.	$\log y = 0.951 \log x + 0.127$

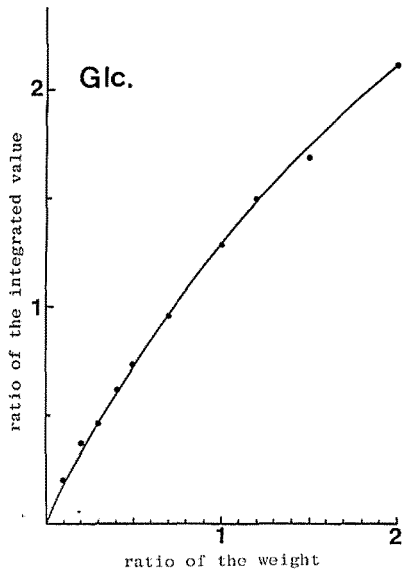

Figure 1. Calibration curve of glucose. 2-Deoxy-D-glucose is used as the internal standard. The numbers on the abscissa indicate the glucose/2-deoxy-D-glucose ratio by the weight, and the numbers on the ordinate the glucose/2-deoxy-D-glucose ratio by the integrated value.

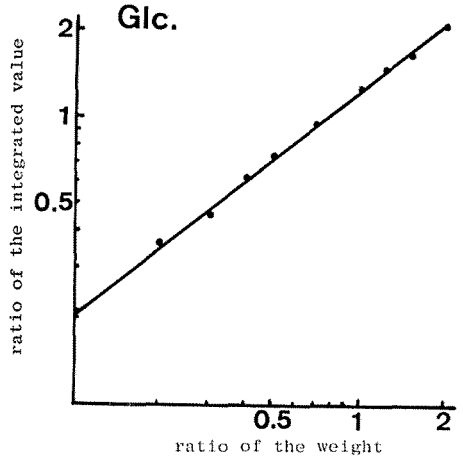

Figure 2. Calibration curve of glucose. The measurements are plotted on log-log graph paper with ratio of the weight on the horizontal axis and ratio of the integrated value on the vertical axis.

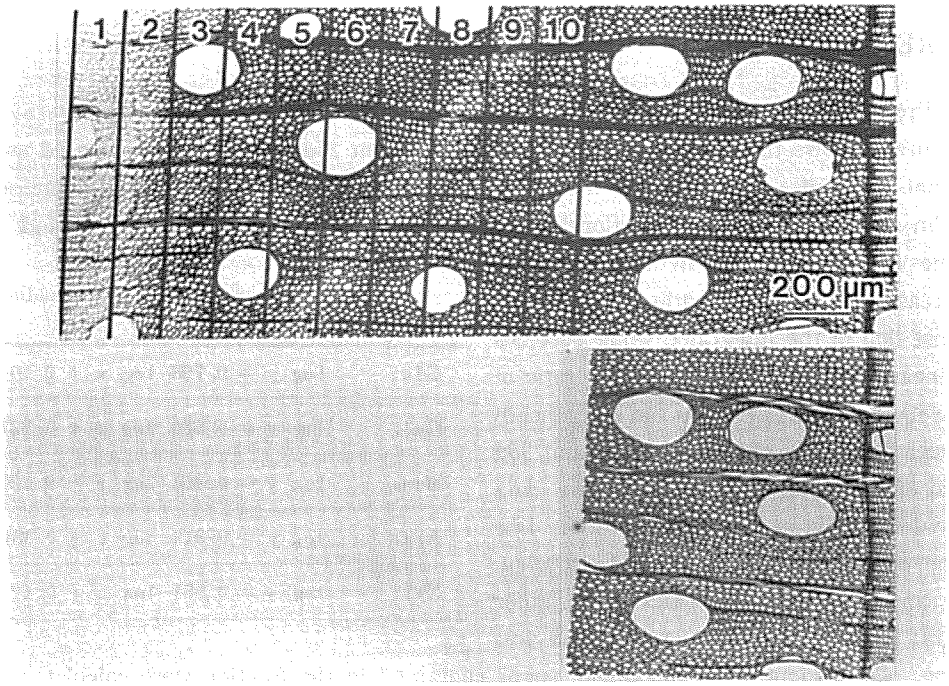

Figure 3. Transverse section of Japanese walnut, which was actually examined for the amount of sugar. The upper photograph shows the transverse section of the specimen block before fractionation, and the lower photograph the transverse section of the specimen block after fractionation. The numbers on the photograph correspond to the fraction number.

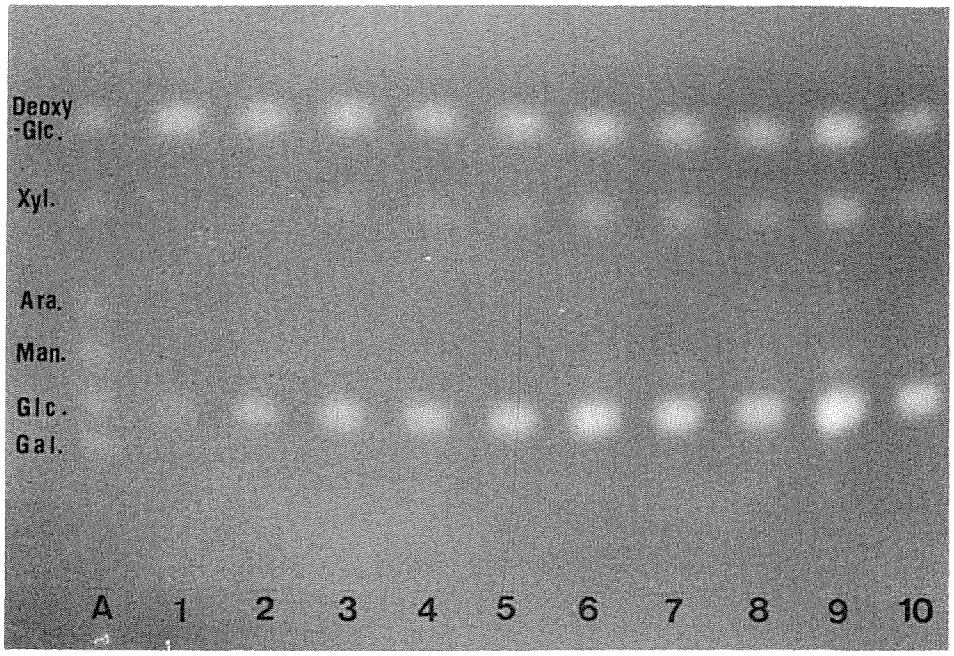

Figure 4. Thin-layer-chromatogram visualized (fluorescence) with ethylenediamine sulfate reagent. Numbers on the chromatogram correspond to the fraction number. Symbols are as follows: A, authentic samples; Deoxy-Glc., 2-deoxy-D-glucose; Xyl., xylose; Ara., arabinose; Man., mannose: Glc., glucose; Gal., galactose.

3.2 Changes in the absolute amount of sugar with the development of Japanese walnut xylem.

Figure 3 shows the transverse section of a specimen used in chemical analysis. The differentiating stages of the fibers in each fraction are as follows.

Fraction 1, primary wall and S_1 formation stage.
Fraction 2, S_1 formation stage.
Fraction 3, S_1 and S_2 formation stage.
Fraction 4-6, S_2 formation stage.
Fraction 7, S_3 formation stage.
Fraction 8-10, probably a stage of secondary wall lignification.

Figure 4 shows the fluorescent light of the spots of TLC-plate, on which hydrolysates were separated, and Figure 5 the absolute amount (corrected value) of sugar contained in each fraction. Glucose continues to inccrease toward Fraction 6, and keeps approximately constant value between Fractions 6 and 10. Xylose increment resembles glucose increment, although the amount of xylose is less than that of glucose. On the other hand, mannose increment is different from increments of other sugars; i.e., mannose increases from Fractions 5 to 7. Arabinose and galactose do not increase during the secondary wall formation.

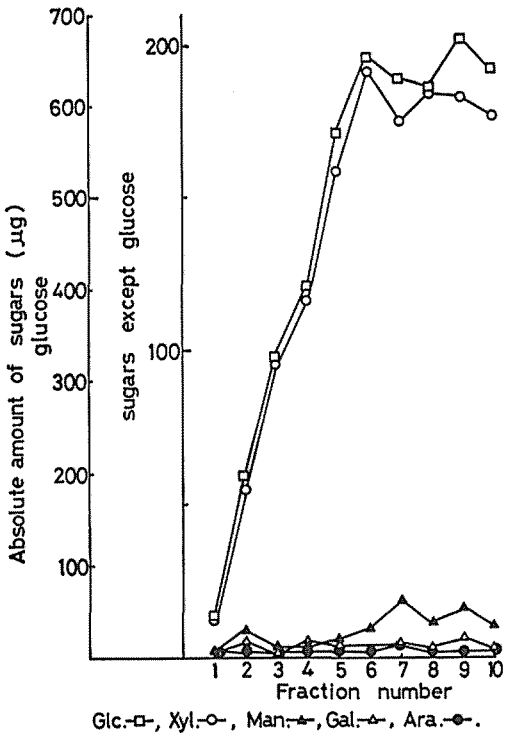

Figure 5. The changes of the absolute amount of sugar accompanied with the xylem development of Japanese walnut.

4. DISCUSSION

The polysaccharides which exsist in the secondary wall of hardwood are cellulose, glucuronoxylan, and glucomannan. Cellulose consists of β-D-glucopyranose residues which are linked by (1-4)-glycosidic bonds. Glucuronoxylan consists of β-D-xylopyranose residues which are linked by (1-4)-glycosidic bonds. The xylose residues in xylan chain carry (1-2)-linked 4-0-methyl-α-D-glucuronic acid residue. Glucomannan consists of β-D-glucopyranose and β-D-mannopyranose, which are linked by (1-4)-glycosidic bonds. These are summarized in Table 2. It is apparent that arabinose and galactose are not present in the polysaccharides of the secondary wall. They are the constituents of hemicelluloses and/or pectin in the primary wall. All of xylose in the secondary wall is derived from glucuronoxylan. On the other hand, glucose is derived from cellulose and glucomannan. Glucomannnan is, however, a minor constituent of polysaccharide in hardwood (only 2-5%

Table 2. Sugar constituents of woody polysaccharides in the secondary wall.

polysaccharide	Glc.	Xyl.	Man.	Ara.	Gal.	GlcU.
cellulose	*					
4−O−methyl−glucuronoxylan		*				*
glucomannan	*		*			

GlcU, 4-O-methyl-glucuronic acid.

of whole polysaccharides in the secondary wall). In addition, mannose/glucose ratio is 1-2. Therefore glucose derived from glucomannan is only 0.7-2.5%. Almost all of the glucose is derived from cellulose. Thus, the amount of glucose, xylose, and mannose were used as indices of the amount of cellulose, glucuronoxylan, and glucomannan, respectively.

The deposition process of polysaccharide with the xylem development of Japanese walnut is discussed as follows. Cellulose deposits continuously from the S_1 stage to the S_3 stage. No increment of glucose in the S_3 stage is probably stemmed from the cell wall structure of hardwood fiber, which has much thinner S_3 layer than tracheid of softwood. Glucuronoxylan deposits in the same manner as cellulose, although the amount of the former is less than that of the latter. On the other hand, the deposition process of glucomannan is different from those of cellulose and glucuronoxylan. The deposition of glucomannan occurs behind the deposition of cellulose and glucuronoxylan; i.e., glucomannan deposits from the later part of S_2 stage to the S_3 stage. This process resembles the deposition process of mannan in cryptomeria. Mannan may play an important role in enhancing lignin affinity in the cell wall. Alternatively, S_3 is possibly rich in mannan. The result that the amount of arabinose and galactose kept constant during the secondary wall formation suggests that hemicelluloses and/or pectin enriched in arabinose and galactose deposit to the primary wall, and remain unchanged during the secondary wall formation.

Acknowledgement The authors thank Associate Professor K. Okamura, Faculty of Agriculture, Kyoto University, for his critical reading of the manuscript, and members of the Wood Structure Laboratory, for their assistance during this study. Part of this work was supported by a Grant-in-Aid for Special Project Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1) Meier, H. and K. C. B. Wilkie: The Distribution of Polysaccharides in the Cell-Wall of Tracheids of Pine (*Pinus silvestris L.*), *Holzforschung* **13**(6), 177-182 (1959)
2) Meier, H.: The Distribution of Polysaccharides in Wood Fibers, *J. Polymer Sci.* **51**, 11-18 (1961)
3) Takabe, K., M. Fujita, H. Harada and H. Saiki: Changes in the Composition and the Absolute Amount of Sugars with the Development of Cryptomeria Tracheids, *Mokuzai Gakkaishi* **29**(3), 183-189 (1983)
4) Iwakawa, J., H. Kobatake, I. Suzuki and H. Kushida: Fluorodensitometric microdetermination of reducing sugars on thin-layer chromatograms, *J. Chromatog.* **193**, 333-337 (1980)
5) Iwakawa, J., H. Kobatake, I. Suzuki and H. Kushida: Densitometric microanalysis of sugars on thin-layer chromatogram, *Bunseki Kagaku* **29**, 441-446 (1980)